Mapping the Peripheral Benzodiazepine Receptor Binding Site by Conformationally Restrained Derivatives of 1-(2-Chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195)

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A synthetic−computational approach to the study of the binding site of peripheral benzodiazepine receptor (PBR) ligands related to 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195, **1**) within their receptor has been developed. A wide series of conformationally restrained derivatives of **1** has been designed with the aim of probing the PBR binding site systematically. The synthesis of these compounds involves palladium-catalyzed coupling and amidation as the key steps. Twenty-nine rigid and semirigid derivatives of **1** were tested in binding studies using [^{3}H]-**1**, and most of these showed PBR affinities in the nanomolar range. The essential role of the carbonyl moiety as a primary pharmacophoric element in the recognition by and the binding to PBR has been confirmed, and the restricted range of the carbonyl orientations, which characterizes the most potent ligands, points to a specific hydrogen-bonding interaction, mainly directed by the geometrical factors, when the electronic ones are fulfilled. Moreover, the fundamental importance of the short-range dispersive interactions in the modulation of the binding affinity and, hence, in the stabilization of the ligand−receptor complex, emerged from the QSAR models reported.

Introduction

The discovery of benzodiazepine binding sites in the periphery (peripheral benzodiazepine receptor, PBR) stimulated intensive research devoted to the characterization of this receptor.[1] Despite these efforts, the physiological role of PBR is still unclear. However, a wide range of pharmacological activities, such as anticonvulsant, anxiolytic, immunomodulating, and cardiovascular, has been related to the activation of this receptor.[2] PK11195 (**1**) is the first non-benzodiazepine ligand which was found to bind the peripheral benzodiazepine receptors (PBR) with nanomolar affinity, and it is now the most commonly used radioligand for this receptor. The structure−affinity relationship data of the analogs of **1** were reported in the patent by Dubroeucq and co-workers and were later discussed by Bourguignon.[3] Georges and co-workers studied a limited set of **1**-like compounds by means of molecular orbital calculations in the attempt to define and map the binding site of **1** in the PBR.[4] However, a comprehensive and systematic investigation of binding site of **1** within PBR is still missing.

The primary structure of PBR is now available,[5] and the information concerning its three-dimensional structure is likely to be sufficient to undertake the determination of the active site of PBR by direct methods in the near future.[6] However, at the present time, this is a very difficult task, and the development of potent ligands must rely on receptor mapping studies by indirect methods. In mapping studies one of the most striking problem is the determination of the ligand conformation bound to the receptor (bioactive conformation); that is why rigid, highly active ligands are the best possible templates.[7]

Chart 1

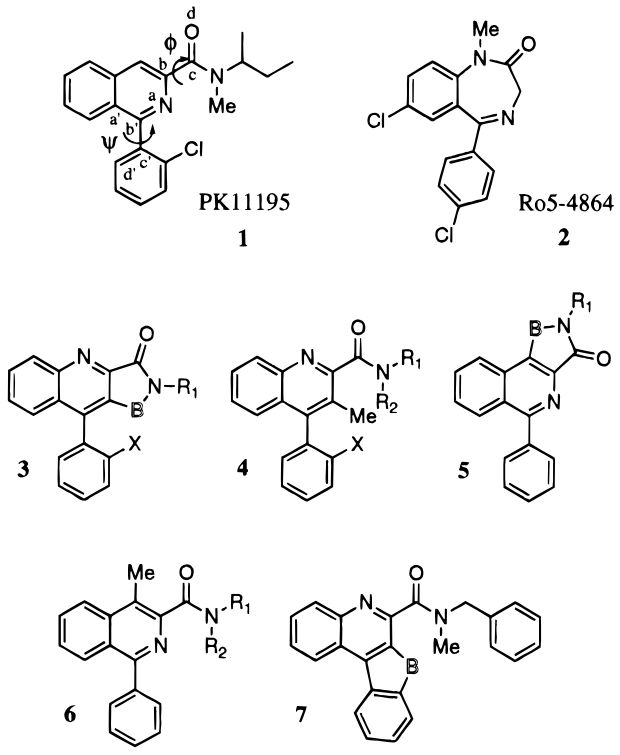

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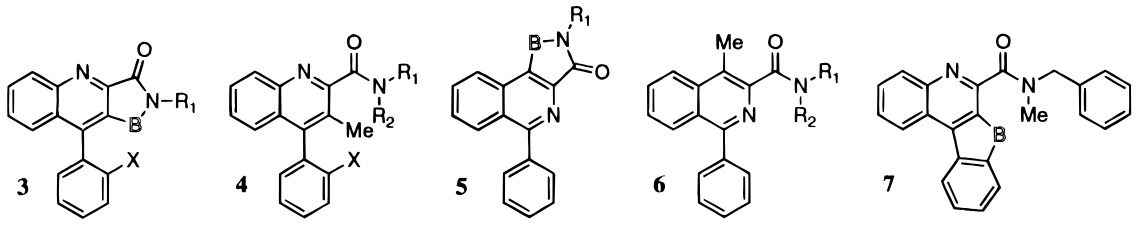
Table 1. Preparative and Analytical Data of Compounds **3**−**7**



compd	bridge (B)	X	R_1	R_2	prep method[a]	yield, %	mp, °C (recryst solv)[b]	formula	anal.[c]
3a	CH_2	H	*s*-Bu		ref 10				
3b	CH_2	F	*s*-Bu		1	81	197−198 (C−EA)	$C_{21}H_{19}N_2OF$	CHN
3c	CH_2	H	Bn		ref 10				
3d	*n*-BuCH	H	Bn		ref 10				
3e	$CH_2CH_2CH_2$	H	Me		ref 11				
3f	$CH_2CH_2CH_2$	H	*s*-Bu		ref 11				
3g	$CH_2CH_2CH_2$	H	Bn		ref 11				
4a		H	*s*-Bu	H	2 A	66	157−158 (H−C)	$C_{21}H_{22}N_2O\cdot{}^1/_8H_2O$	CHN
4b		F	*s*-Bu	H	2 A	75	161−162 (H−C)	$C_{21}H_{21}N_2OF$	CHN
4c		H	Bn	H	2 A	81	127−128 (H−C)	$C_{24}H_{20}N_2O\cdot{}^1/_8H_2O$	CHN
4d		H	4-ClBn	H	2 A	76	123−124 (H−C)	$C_{24}H_{19}N_2OCl$	CHN
4e		F	4-ClBn	H	2 A	87	131−133 (C−H)	$C_{24}H_{18}N_2OClF$	CHN
4f		H	*s*-Bu	Me	2 B	64	117−118 (H)	$C_{22}H_{24}N_2O\cdot{}^1/_4H_2O$	CHN
4g		F	*s*-Bu	Me	2 B	87	oil	$C_{22}H_{23}N_2OF$	CHN
4h		H	Bn	Me	2 C	84	118−120	$C_{25}H_{22}N_2O\cdot{}^1/_4H_2O$	CHN
4i		H	4-ClBn	Me	2 B	66	132−133 (H−C)	$C_{25}H_{21}N_2OCl$	CHN
4j		F	4-ClBn	Me	2 B	81	107−108	$C_{25}H_{20}N_2OClF$	CHN
4k		H	4-ClPh	Me	2 C	72	165−166 (C−EA)	$C_{24}H_{19}N_2OCl$	CHN
4l		H	4-MeOPh	Me	2 C	64	133−134 (H−EA)	$C_{25}H_{22}N_2O_2$	CHN
5a	CH_2		*s*-Bu		3	90	193−194 (EA−C)	$C_{21}H_{20}N_2O$	CHN
5b	CH_2		Bn		3	95	243−244 (EA−CH)	$C_{24}H_{18}N_2O$	CHN
5c	$CH_2CH_2CH_2$		*s*-Bu		4 D	64	153−154 (EA−H)	$C_{23}H_{24}N_2O$	CHN
5d	$CH{=}CHCH_2$[d]		*s*-Bu		4 D	65	193−194 (EA)	$C_{23}H_{22}N_2O$	CHN
5e	$CH{=}CHCH_2$[d]		Bn		4 E	98	179−180 (EA)	$C_{26}H_{20}N_2O$	CHN
6a			*s*-Bu	H	5 A	86	120−121 (H)	$C_{21}H_{22}N_2O$	CHN
6b			*s*-Bu	Me	5 B	84	136−137	$C_{22}H_{24}N_2O$	CHN
6c			Bn	Me	5 C	86	oil	$C_{25}H_{22}N_2O$	CHN
7a	CH_2CH_2				6 F	59	oil	$C_{26}H_{22}N_2O\cdot{}^{1/4}H_2O$	CHN
7b	OCH_2CH_2[e]				6 F	82	154−155	$C_{26}H_{22}N_2O_2$	CHN

[a] In the preparation method the number indicates the scheme and the letter the method for the newly synthesized compounds, while references are reported for the known compounds. [b] Recrystallization solvents; EA, ethyl acetate; H, *n*-hexane; C, cyclohexane; CH, chloroform. [c] Analyses for the elements indicated were within ±0.4% of the theoretical values. [d] See Scheme 4. [e] See Scheme 6.

In the large amount of PBR ligands developed up to now the most rigid high-affinity ligand is Ro5-4864 (**2**), while **1** shows at least one important degree of conformational freedom.[4] These two ligands are supposed to bind nonidentical binding domains, as they have been reported to have different binding modalities.[6a]

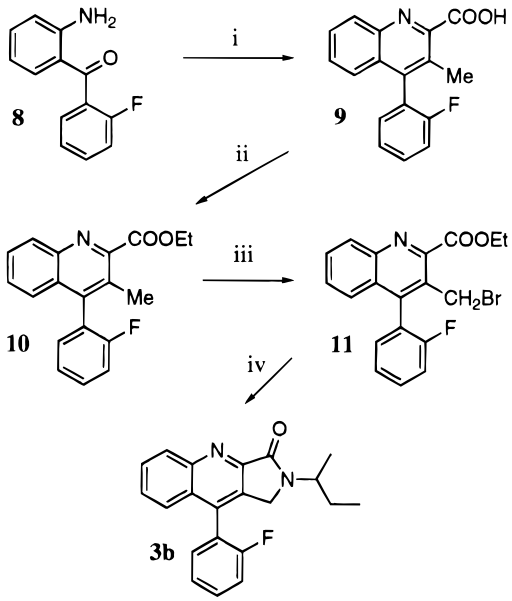
In this paper we present a new systematic approach to the study of the binding site of **1**-like compounds within PBR, based on the design of conformationally constrained derivatives **3**−**7**.

Moreover, electronic indexes[8] as well as ad-hoc-defined size and shape molecular descriptors[9] have been considered and employed in order to decipher, on the basis of the complementarity principle, the binding site features on quantitative grounds.

Chemistry

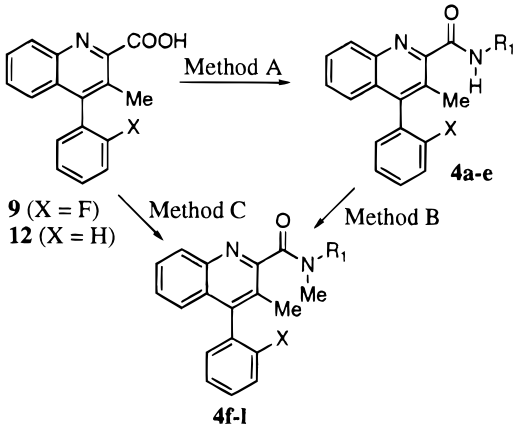
The synthesis of pyrrolo[3,4-*b*]quinolin-3-ones **3a,c,d** has recently been published by some of us,[10] and the related compound **3b** (Table 1) was prepared by using the reported methodology starting from 2′-fluorobenzophenone **8** (Scheme 1). Friedländer condensation (base-catalyzed) of **8** with 2-oxobutyric acid gave **9**, which was characterized by ^{1}H-NMR spectroscopy and transformed into the corresponding ethyl ester **10**. Bromination of **10** with NBS in the presence of dibenzoyl peroxide gave γ-bromo ester **11**, which was converted into γ-lactam **3b** by using *sec*-butylamine in ethanol at reflux in the presence of K_2CO_3.

Scheme 1[a]



[a] Reagents: (i) EtCOCOOH, MeONa, MeOH; (ii) $POCl_3$, EtOH; (iii) NBS, (PhCOO)2, CCl4; (iv) *s*-$BuNH_2$, K_2CO_3, EtOH.

Scheme 2



The preparation of azepino[3,4-*b*]quinolin-5-ones **3e,f,g** is the object of a recent publication by some of us.[11]

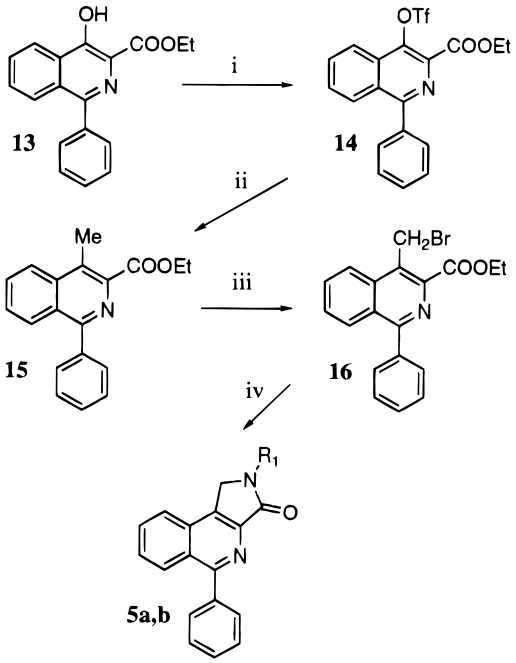
Secondary amides **4a**−**e** (Scheme 2) were prepared by reaction of the acid chloride generated from the suitable carboxylic acids **9** or **12**[10] with an appropriate primary amine (method A) and then converted into the corresponding tertiary amides by alkylating the amide anion (generated with NaH) using MeI (method B). Tertiary amide **4h** was prepared by reaction of the chloride of acid **12** with *N*-methylbenzylamine (method C).

The synthesis of all the compounds containing the isoquinoline core was rationalized through the search for a common intermediate, and aryl triflate **14** was chosen as the starting material. This compound was prepared from the corresponding enolate **13**[12] by reaction with triflic anhydride in pyridine. Aryl triflate **14** underwent Stille coupling[13] with tetramethyltin to give the corresponding methyl derivative **15** in very good yield. Compound **15** was brominated in its benzyl position with NBS in CCl_4 in the presence of a radical initiator (dibenzoyl peroxide), and it was then reacted with the suitable primary amine to give pyrroloisoquinolines **5a,b** (Scheme 3).

The synthesis of the compounds containing the new azepino[3,4-*c*]isoquinoline ring system is shown in Scheme 4. Aryl triflate **14** underwent palladium-catalyzed coupling with propargyl alcohol[14] in the presence of CuI and Et_3N to give compound **17** in good yield. Catalytic reduction of **17** in the presence of Pd/C gave saturated alcohol **18**. This was chlorinated with $SOCl_2$, allowed to react with *sec*-butylamine, and then cyclized with the procedure previously described for the synthesis of compound **3f**[11] (method D). Alternatively, compound **17** was hydrogenated in the presence of PdS on carbon to give allylic alcohol **19** the *Z* geometry of which at the double bond was confirmed by ^{1}H-NMR spectroscopy (J = 11.3 Hz for the vinylic hydrogens in DMSO). Alcohol **19** was transformed into **5d** by using method D, or reacted first with $SOCl_2$, and then with benzylamine, and finally, cyclized in toluene in the presence of 2-hydroxypyridine to give azepinoisoquinoline **5e** (method E).

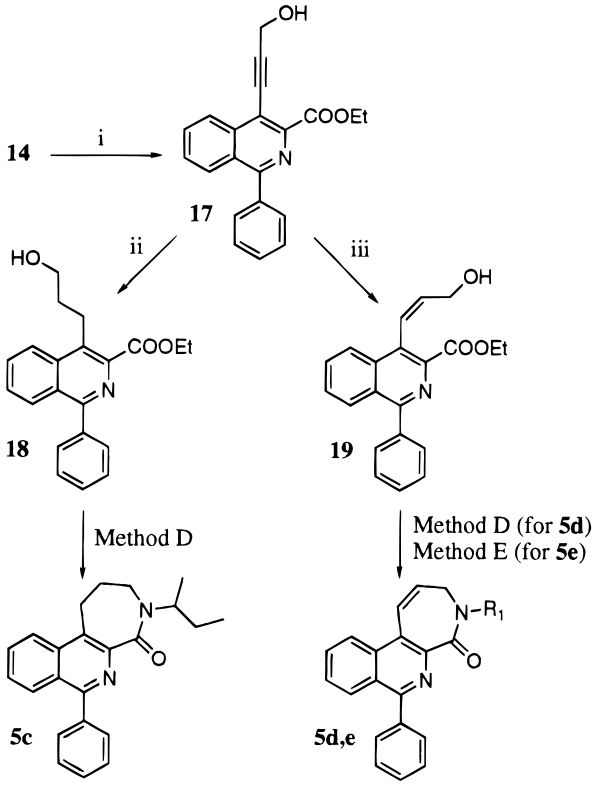
Hydrolysis of ester **15** gave carboxylic acid **20**, which was promptly converted into secondary amide **6a** by using method A or into tertiary amide **6c** by using method C (Scheme 5). The alkylation of secondary amide **6a** (method B) gave tertiary amide **6b**.

Scheme 3[a]



[a] Reagents: (i) Tf_2O, Py; (ii) Me_4Sn, LiCl, $Pd(PPh_3)_2Cl_2$, DMF; (iii) NBS, $(PhCOO)_2$, CCl_4; (iv) R_1NH_2, EtOH.

Scheme 4[a]



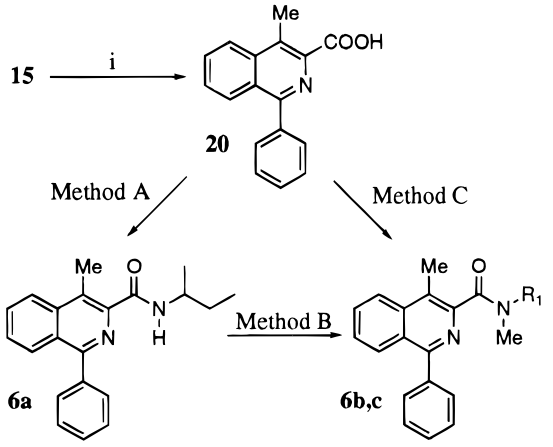
[a] Reagents: (i) $HC{\equiv}CCH_2OH$, CuI, $Pd(PPH_3)_2Cl_2$, Et_3N, DMF; (ii) H_2, Pd/C, EtOH; (iii) H_2, PdS/C, EtOH.

Finally, the compounds with the locked phenyl group **7a,b** were synthesized by the palladium-catalyzed amidation[15] of the corresponding imidoyl chlorides **21a** or **21b** (Scheme 6).[16]

Results and Discussion

Determination of the Bioactive Conformation. The importance of the carbonyl group in the interaction

Scheme 5[a]



[a] Reagents: (i) NaOH, EtOH.

of PBR ligands with their receptors has been stressed by several authors.[3,4,17,18] This is one of the most important groups in the structure of **1**, which is, in principle, capable of giving long-range (strong and directional) electrostatic interactions with the receptor.

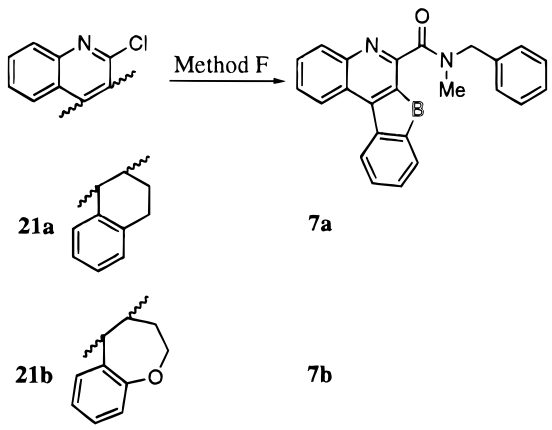
With the aim of systematically probing the PBR bound conformation, a series of conformationally constrained derivatives of **1**, in which the orientation of the carbonyl dipole is suitably spaced around its degree of conformational freedom, were designed. The 10-π aromatic system (quinoline or isoquinoline) with the pendant phenyl ring, typical of the structure of **1**, was preserved in all the new compounds and provides the reference frame for the rotation of the carbonyl dipole around the bond linking to the aromatic system (ϕ = dihedral angle between the plane of the bicyclic aromatic system and the plane of the carbonyl group (a−b−c−d), see Chart 1).

All the compounds belonging to this series of conformationally constrained derivatives of **1** were tested for their potential ability to displace [^{3}H]-**1** from PBR in rat cortex in comparison with **1**, and the results of these studies are summarized in Table 2. The values of the dihedral angle ϕ found in the AM1[19] lowest minimum energy conformers of compounds **3**−**7** are also tabulated for comparative purposes.

From these results it appears that tertiary amides **4f**−**l**, **6b,c**, and **7a,b** inhibited [^{3}H]-**1** binding, showing IC_{50} values in the low nanomolar range and very similar to that shown by **1**. In these compounds the steric repulsion between the amide methyl group and the 10-π aromatic system methyl substituent (or the bridge methylene in the case of **7a,b**) makes a large portion of conformational space energetically inaccessible. This is shown in Figure 1, where the AM1 energy profiles of the parent compounds **4g** and **6b** are compared with the energy profile of compound **1**.

The AM1 absolute minimum, which corresponds to ϕ = 110° and 112° in compound **1** and **6b**, respectively, shifts to 85° in compound **4g**. Moreover, the presence of the methyl substituents on the 10-π aromatic system significantly reduces the breadth of the minimum, as expected. In fact, for compound **1** the conformations found within 3 kcal/mol from the global minimum correspond to dihedral angle values between the plane of the bicyclic aromatic system and the carbonyl axis in the range of 70° < ϕ < 140°, whereas the range is

Scheme 6



reduced to 60° < ϕ < 110° for compound **4g**, and to 80° < ϕ < 130° for compound **6b**. A good agreement is observed between the range of allowed conformations ($\Delta E \leq 3$ kcal/mol) obtained for these compounds by means of the AM1 semiempirical method and the *ab initio* results at the 6-31G* level.[20] The results are summarized in Table 3.

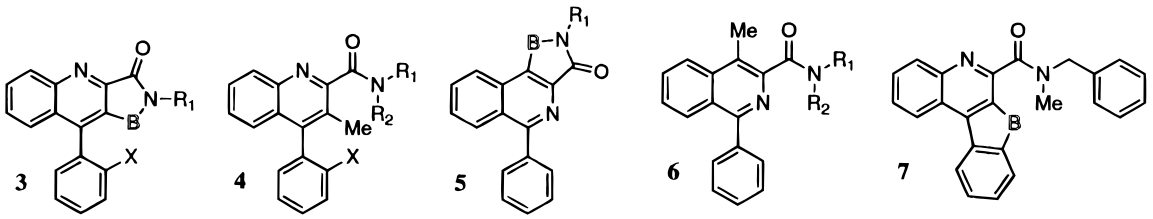
If the same binding modalities are assumed for compounds **4f**−**l, 6b,c**, and **7a,b**, these should bind PBR with the same carbonyl orientations. Following this idea, the high affinity of compounds **4f-l**, **6b-c**, and **7a,b** suggests that the conformational space shared by the carbonyl moieties of compounds **4f**−**l, 6b,c**, and **7a,b** (corresponding to the carbonyl orientations comprised in the range 80° < ϕ < 110°) defines the orientation in which the amide carbonyl of the PBR ligands related to **1** interacts with a suitable amino acid residue in the receptor. In other words, the high-affinity PBR ligands related to **1** interact with their receptor, showing nearly perpendicular carbonyl orientations with respect to the plane of the bicyclic aromatic system.[4]

The low affinities showed by the constrained derivatives **3a**−**g** and **5a**−**e** strongly support this hypothesis (see Table 2). In fact, compounds **3a**−**d**, in which the carbonyl dipole is locked on the same plane as the bicyclic aromatic system and shows a trans orientation with respect to the pendant phenyl ring ($\phi \approx 180°$), are essentially inactive. In the case of compounds **3e**−**g**, PBR affinity significantly increases along with the increasing size of the amide substituent, but it never reaches the low nanomolar level. The AM1-optimized structure of such compounds shows that the carbonyl group tends to be oriented out of the plane of the bicylic aromatic system with a dihedral angle $\phi \approx 120°$. Thus, the carbonyl orientation of compounds **3e**−**g** approaches the assumed bioactive range (80° < ϕ < 110°), and it is noteworthy that compound **3g** is >59 times more active than **3c** ($\phi \approx 180°$) and 81 times less active than **4h** (ϕ = 85°). A similar consideration also applies to compounds **5c**−**e** for which X-ray diffraction studies performed on **5d** (see the Experimental Section) showed that the dihedral angle ϕ was about 43° and relaxed to 51° after AM1 full optimization.

It is interesting to note that compound **5e** is only about 1 order of magnitude less potent than **1** and about 1 order of magnitude more active than compound **5b** where the carbonyl dipole is locked in the planar cis orientation ($\phi \approx 0°$).

Secondary amides **4a**−**e** and **6a** show significantly lower PBR affinities when compared to their N-methy-

Table 2. PBR Binding Affinities and ϕ Dihedral Angle Values of the AM1 Lowest Minimum Energy Conformers of Compounds **3**−**7**



compd	bridge (B)	X	R_1	R_2	IC_{50} (nM) ± SEM[a]	ϕ (deg)[b]
3a	CH_2	H	*s*-Bu		>10000	175
3b	CH_2	F	*s*-Bu		>10000	175
3c	CH_2	H	Bn		>10000	175
3d	*n*-BuCH	H	Bn		810 ± 110	175
3e	$CH_2CH_2CH_2$	H	Me		>10000	122
3f	$CH_2CH_2CH_2$	H	*s*-Bu		1200 ± 200	122
3g	$CH_2CH_2CH_2$	H	Bn		170 ± 14	122
4a		H	*s*-Bu	H	230 ± 70	64
4b		F	*s*-Bu	H	13 ± 4.9	64
4c		H	Bn	H	1200 ± 180	64
4d		H	4-ClBn	H	1700 ± 230	64
4e		F	4-ClBn	H	270 ± 66	64
4f		H	*s*-Bu	Me	2.1 ± 0.9	85
4g		F	*s*-Bu	Me	2.9 ± 0.5	85
4h		H	Bn	Me	2.1 ± 0.6	85
4i		H	4-ClBn	Me	9.8 ± 2.0	85
4j		F	4-ClBn	Me	3.4 ± 1.0	85
4k		H	4-ClPh	Me	6.4 ± 1.1	85
4l		H	4-MeO-Ph	Me	8.8 ± 1.5	85
5a	CH_2		*s*-Bu		620 ± 100	2
5b	CH_2		Bn		480 ± 29	2
5c	$CH_2CH_2CH_2$		*s*-Bu		780 ± 20	51
5d	$CH{=}CHCH_2$[c]		*s*-Bu		490 ± 47	51
5e	$CH{=}CHCH_2$[c]		Bn		45 ± 15	51
6a			*s*-Bu	H	550 ± 93	132
6b			*s*-Bu	Me	11 ± 2.6	112
6c			Bn	Me	3.1 ± 0.8	112
7a	CH_2CH_2				8.9 ± 2.5	85
7b	OCH_2CH_2[d]				10 ± 0.7	85
1					2.2 ± 0.3	110

[a] Each value is the mean ± SEM of three determinations. [b] ϕ is the dihedral angle between the plane of the bicyclic aromatic system and the plane of the carbonyl group (a−b−c−d), see Chart 1. [c] See Scheme 4. [d] See Scheme 6.

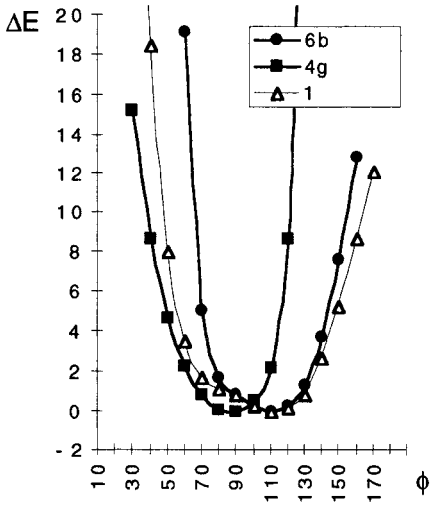


Figure 1. Conformational energy ΔE (kcal/mol) *vs* dihedral angle ϕ for compounds **4g** and **6b**. The energy profile of compound **1** (PK11195) is reported for comparative purposes.

lated counterparts **4f**−**j** and **6b**. These differences in PBR binding affinity appear to be very difficult to rationalize when the conformational profile of these compounds (Figure 2) is compared to those reported for compounds **4g** and **6b** (Figure 1). In fact, only a moderate shift and broadening of the minimum in the region comprised between 40° < ϕ < 110° for compound **4b**, and 80° < ϕ < 160° for compound **6a** is observed. On the other hand, the differences in the conformational preferences between secondary and tertiary amides are amplified by analyzing the range of allowed conformations at the *ab initio* level (Table 3), since this analysis shows that the minimum comprises ϕ dihedral angle values in the range 0−80° (compound **4b**) and 90−180° (compound **6a**). However, these data also appear to be inadequate to completely account for the relevant differences in PBR affinity between secondary and tertiary amides, so further work is in progress in order to explain this intriguing finding.

Along with the amide carbonyl, the pendant phenyl is a pharmacophoric group[3,4,18] in the structure of **1** which presents 1 degree of conformational freedom. To determine the bioactive conformation of this phenyl ring, 6-31G* calculations were performed on the most active compound **4h**. The strong steric repulsion between the hydrogen atoms in the 2,6-positions of the phenyl group and that in 5-position of the bicyclic system or those of the nuclear methyl group was found to constrain the orientation of the phenyl moiety out of the plane of the bicyclic system. Thus, for compound **4h** the lowest energy minimum conformation shows a dihedral angle between the bicyclic system and the phenyl substituent (a′−b′−c′−d′) (see Chart 1) $\psi = 90°$, the energetically allowed phenyl orientations (within 3 kcal/mol from the minimum) are in the range of 60° <

Table 3. Comparison between AM1 and *ab Initio* (RHF/6-31G*) Conformational Preferences of Some Selected Compounds

		AM1		6-31G*		
compd	pIC_{50}	ϕ min[a] (deg)	range of conf within 3 kcal/mol[b] (deg)	ϕ min[a] (deg)	range of conf within 3 kcal/mol[b] (deg)	ΔE[c] (kcal/mol)
4g	8.54	85	60−110	70	60−90	0.06
6b	7.95	112	80−130	112	90−130	0.00
4b	7.89	64	40−110	30	0−80	1.41
6a	6.26	132	80−160	132	90−180	0.00
1	8.66	110	70−140	110	80−150	0.00

[a] ϕ min is the dihedral angle value of the lowest minimum energy conformer. [b] Range of ϕ values in the conformers within 3 kcal/mol from the lowest energy minimum. [c] ΔE is the energy difference, computed at *ab initio* level, between the conformer corrresponding to the 6-31G* absolute minimum and the conformer corresponding to the AM1 absolute minimum.

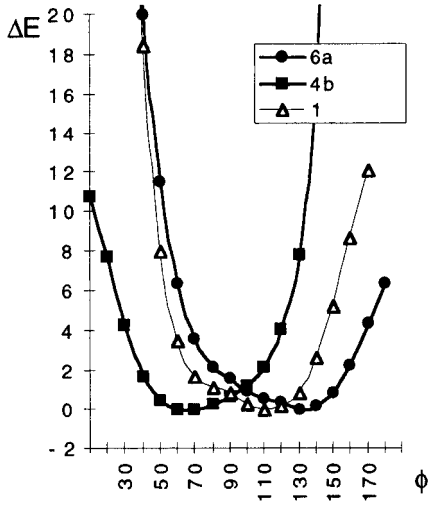


Figure 2. Conformational energy ΔE (kcal/mol) *vs* dihedral angle ϕ for compounds **4b** and **6a**. The energy profile of compound **1** (PK11195) is reported for comparative purposes.

$\psi < 110°$, and the conformation showing $\psi = 40°$ is 39 kcal/mol less stable than the minimum. On the other hand, compound **7a** shows a dihedral angle $\psi = 27°$ and is slightly less active than **4h**, while **7b** shows both a dihedral angle $\psi = 50°$ and a PBR affinity comparable to that of **7a**. Therefore, the (quasi systematic) constraining of the pendant phenyl group fails to reveal a clear preference of the receptor in the interaction with a particular conformation of the phenyl. Thus, the orientation of the phenyl substituent does not appear to be of crucial importance for the interaction of PBR ligands related to **1** with their receptor, according to the hypothesis by Georges et al.[4]

Qualitative Structure−Affinity Relationships. Although the remarkable structural similarity existing in all the new compounds might be mirrored by a similarity in the nature of the ligand−receptor interactions for all the constrained derivatives, a structure−affinity relationship (SAFIR) study has been undertaken in order to test this assumption. Initially, the role of the amide substituents was characterized by introducing several highly lipophilic side chains on compounds **4**. The results showed that both benzyl and *sec*-butyl groups are optimal for the interaction with the receptor despite their structural differences. These two amide substituents were then selected for the characterization of similar interactions with the PBR binding site of the remaining new compounds **3**, **5**, **6**, and in most cases the benzyl group showed higher affinity than the *sec*-butyl. It is interesting to remark that the positive influence of the benzyl group is more evident in those compounds provided with medium to low affinity (compare **3f** with **3g**, and **5d** with **5e**) and it is reduced or absent in compounds with high affinity (compare **6b** with **6c**, and **4f** with **4h**). This particular behavior seems to claim for a short-range attractive interaction sustained by the amide side chain, and the homogeneous SAFIR trend suggests that a common mode of interaction with the receptor exists.

A similar SAFIR trend was found when the effect of the 2′-substitution in compounds **4** was investigated. In fact, the positive influence of the 2′-fluoro substitution is more evident in compounds with medium to low affinity and is reduced or absent in compounds with high affinity. It is noteworthy that the effects of the 2′-fluoro substitution on the PBR affinities of compounds **4a**−**j** are very similar to those described for the 2′-chloro substitution in analogous isoquinolines (see ref 3), suggesting for the alogen in 2′-position a hydrophobic role. Since for the pendant phenyl group a short-range attractive interaction is feasible,[3,4] a further characterization of the kind of interaction sustained by the pendant phenyl group was obtained by the conformational constraining of the phenyl ring in different space positions as it occurs in compounds **7a** and **7b**. The lack of strict structural requirements for the binding of the phenyl ring (see the previous section) suggests that the ring is likely to perform no more than a hydrophobic space-filling role.

Finally, the position and role of the nitrogen atoms in the quinoline and isoquinoline nuclei seem to be irrelevant.

Quantitative Structure−Affinity Relationships. A quantitative rationalization of this SAFIR study was attempted by using electronic indexes and size and shape descriptors derived by molecular modeling. A large set of molecular orbital derived electronic reactivity indexes[8] was calculated in the AM1 framework for the whole series of compounds reported in Table 2, and at *ab initio* level (RHF/6-31G*) for a selected subset of representative compounds. Table 4 lists some selected electronic indexes computed with the semiempirical AM1 hamiltonian and with 6-31G* basis set. It can be noted that in average 6-31G* charges on the carbonylic oxygen atom (qO), derived from Mulliken population analysis, are greater in magnitude than the corresponding AM1 charges, but the scaling factors vary very slightly from one molecule to another; therefore, a good correlation ($r = 0.974$) is observed between the variation of the AM1 and 6-31G* charges along the series of compounds considered. The same holds for the energy of the highest occupied molecular orbital (E_{HOMO}) ($r = 0.986$) and for the dipole moment of the molecules (μ) ($r = 0.973$).

An accurate correlation analysis between the electronic and reactivity indexes and PBR binding affinity

Table 4. Comparison between *ab Initio* (RHF/6-31G*) and AM1 Molecular Orbital Indices Computed on a Representative Subset of Compounds

		6-31G*			AM1		
compd	pIC_{50}	qO[a]	E_{HOMO}[b] (eV)	μ[c] (Debye)	qO[a]	E_{HOMO}[b] (eV)	μ[c] (Debye)
3a	NA[d]	−0.6182	−8.3561	6.9662	−0.3075	−9.1942	5.6900
3g	6.77	−0.6263	−8.2439	6.0407	−0.3397	−9.1561	5.0586
4a	6.64	−0.6314	−8.2347	3.3310	−0.3649	−9.1053	2.8126
4b	7.89	−0.6323	−8.2688	3.9540	−0.3563	−9.1724	3.2837
4f	8.68	−0.6292	−8.2106	4.9731	−0.3496	−9.1178	4.2415
4g	8.54	−0.6294	−8.2106	4.9731	−0.3509	−9.1571	3.8818
4h	8.68	−0.6350	−8.2372	4.8556	−0.3544	−9.1398	4.2700
4i	8.01	−0.6353	−8.2984	4.9776	−0.3523	−9.1818	4.2623
5b	6.32	−0.5391	−8.2439	6.1256	−0.2658	−9.1244	4.9706
5d	6.31	−0.5317	−7.6954	4.2840	−0.2797	−8.7829	3.5042
6a	6.26	−0.6438	−7.9351	4.0953	−0.3765	−8.9230	3.1811
6b	7.95	−0.6395	−7.9349	4.3953	−0.3630	−8.9305	3.7329
7a	8.05	−0.6351	−7.9137	4.9281	−0.3524	−8.8738	4.3871
1	8.66	−0.6346	−8.1424	4.0784	−0.3616	−9.0930	3.3748

[a] qO is the charge on the carbonylic oxygen atom. [b] E_{HOMO} is the energy of the highest occupied molecular orbital. [c] μ is the dipole moment of the molecule. [d] NA = not active (see Table 2).

Table 5. PBR Binding Affinities (pIC_{50}) and Ad-Hoc-Defined Size and Shape Descriptors (V_d, V_{in}, V_{out}, d, and α) of Conformationally Restrained Derivatives of **1**

compd	pIC_{50}	V_d	V_{in} (Å^3)	V_{out} (Å^3)	d (Å)	α (deg)
3d	6.09	0.43	274.00	86.38	8.14	−165.50
3f	5.92	0.54	270.38	37.50	6.93	−171.40
3g	6.77	0.52	277.63	50.75	7.75	−141.20
4a	6.64	0.49	250.88	38.12	6.50	−156.40
4b	7.89	0.62	282.50	11.63	6.99	−153.40
4c	5.92	0.46	253.25	53.00	7.86	−138.00
4d	5.77	0.43	252.75	64.25	7.86	−138.00
4e	6.57	0.50	269.38	52.00	7.84	−145.10
4f	8.68	0.70	304.00	0.00	7.07	−158.30
4g	8.54	0.69	304.75	4.88	7.07	−158.30
4h	8.68	0.74	322.63	0.00	8.09	−163.50
4i	8.01	0.66	308.88	23.75	8.20	−155.10
4j	8.47	0.67	314.88	23.75	8.12	−157.90
4k	8.19	0.59	287.75	29.50	7.58	−167.50
4l	8.06	0.57	288.00	40.63	7.56	−167.10
5a	6.21	0.44	234.88	42.87	5.96	−154.20
5b	6.32	0.45	246.50	50.13	7.36	−139.00
5c	6.10	0.53	270.88	38.62	6.75	−168.30
5d	6.31	0.53	268.25	35.25	6.45	−141.50
5e	7.34	0.55	280.50	42.75	6.64	−159.60
6a	6.26	0.51	255.63	31.50	7.00	−179.30
6b	7.95	0.65	294.50	10.13	7.18	−165.70
6c	8.51	0.74	321.50	0.00	8.00	−168.40
7a	8.05	0.60	291.88	31.62	7.86	−163.60
7b	8.00	0.63	304.50	28.88	7.85	−164.20
1	8.66	0.62	285.00	13.75	7.19	−161.50

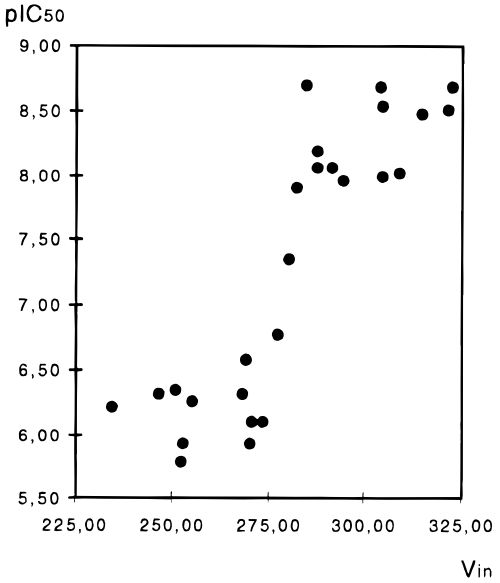


Figure 3. Correlation between the PBR binding affinities data values (pIC_{50}) and the data values of the van der Waals volume included (V_{in}) in the reference volume of the supermolecule (see text) for the ligands listed in Table 5. The linear regression equation is $pIC_{50} = 0.04(\pm0.001)V_{in} - 3.62(\pm1.29)$, $n = 26$, $r = 0.872$, $s = 0.551$, $F = 71.92$, where n is the number of compounds, r the correlation coefficient, s the standard deviation, and F the value of the Fisher ratio; the numbers in parentheses are the 95% confidence intervals of the regression coefficient and of the intercept.

did not reveal any organized trend (data not reported). This result suggests that, while the electronic features of the ligands considered, and, in particular, those localized on the high polar carbonyl group, are essential for the recognition of the PBR, they are unable to describe quantitatively the variation of the ligands affinity toward the PBR. However, as previously shown for molecular series of protonated ligands which bind G-protein-coupled receptor[9,21] the modulation of the ligand binding affinity is explained by the "ad hoc" defined size and shape descriptors.

Table 5 reports some selected geometrical as well as size and shape molecular descriptors together with the cologarithmic form of the considered ligands' binding affinity values.

We assume that the volume obtained by superimposing the most structurally different ligands showing the highest affinities reflects the overall shape and the conformational freedom of the PBR binding site. Thus, compounds **4f**, **4h**, and **6c** were chosen to constitute the supermolecule and were superimposed as described in the computational procedure section. Molecular descriptors V_{in} and V_{out} are, respectively, the inner and the outer van der Waals molecular volumes of the ligands considered in relation to the resultant reference volume of the supermolecule, and from an interpretative point of view, they mimic the short-range attractive and repulsive interactions with the receptor, respectively.[9] V_d is defined as the difference between V_{in} and V_{out} and is normalized with respect to the volume of the supermolecule. The most significant QSAR models obtained are shown in Figures 3 and 4, where V_{in} and V_d are plotted against the PBR binding affinity data values. The higher the molecular volume shared by a generic ligand and the supermolecule (V_{in}), the higher the binding affinity. A positive slope is also obtained in the correlation involving V_d. Recalling the mathematical formulation of this molecular descriptor, the correlation

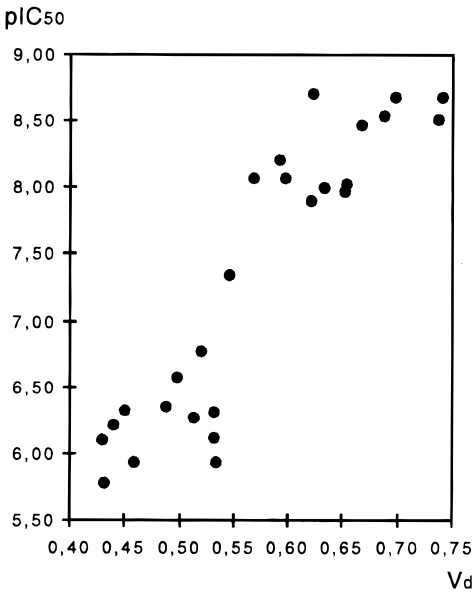


Figure 4. Correlation between the PBR binding affinities data values (pIC_{50}) and the data values of the $V_d = (V_{in} - V_{out})/V^{sup}$ for the ligands listed in Table 5. The linear regression equation is $pIC_{50} = 10.25(\pm 0.97)V_d + 1.43(\pm 0.56)$, $n = 26$, $r = 0.912$, $s = 0.462$, $F = 111.09$.

suggests that, in the molecular series of the compounds considered, the binding affinities are modulated by the molecular shape of the amide substituents through the optimization of dispersive and steric interactions.

The need for severe steric binding requirements is also supported by a qualitative inspection of the last two columns of Table 5, which shows (a) the distance (d) between the centroid of the 10-π aromatic system and the centroid of the amidic substituents and (b) the dihedral angle (α) which defines the spatial orientation of the main plane on which the amidic substituents lie with respect to the bicyclic aromatic system.

The data values of these two geometrical descriptors seem to indicate that in order to obtain high binding affinities in this molecular series of ligands the two conditions $d > 7$ Å and $-168° < \alpha < -153°$ have to be satisfied simultaneously.

Any attempt to improve the linear QSAR models presented by multiregression analysis of the above mentioned size and shape descriptors with a large variety of electronic and reactivity indexes[8] was unsuccessful. These elements taken together seem to support the hypothesis that specific highly directional interaction with a suitable hydrogen-bonding donor amino acid residue of the receptor can easily be achieved from the point of view of the electronic requirements (being the electronic character of the CO group quite similar in the ligands considered), once the conformational requirements are satisfied.

Conclusions

A combined synthetic−computational approach to the receptor mapping has been applied and a large series of conformationally constrained derivatives of **1** has been investigated in order to probe the PBR binding site in a systematic way. On this basis, structural, conformational, and electronic requirements for the binding are proposed. The essential role of the carbonyl function as a primary pharmacophoric element in the recognition by and the binding to PBR has been confirmed and the restricted range of carbonyl orientations (comprised between 80° and 110°) which characterizes the ligands with the greatest affinity implies a specific hydrogen-bonding interaction. A second important pharmacophoric element has emerged from the quantitative structure−activity relationship (QSAR) models obtained and concerns the amidic substituents. In fact, ad-hoc-defined size and shape descriptors have proved to be able to rationalize the variation in PBR binding affinity quantitatively. They are assumed to interpret the variation of the short-range intermolecular interaction in the ligand−receptor complex. The pharmacophoric function of the pendant phenyl ring is essential, although its constraining fails to reveal a clear preference of the receptor in interacting with a particular conformation. Thus, the lack of strict structural requirements for the binding of the phenyl group suggests a dispersive nature of its interaction with the receptor. For the fluorine atom in the 2′-position a hydrophobic role may be suggested. Finally, the position and role of the nitrogen atoms in the quinoline and isoquinoline nuclei seem to be irrelevant.

Experimental Section

Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Microanalyses were carried out using a Perkin-Elmer 240C elemental analyzer. Merck silica gel 60 (70−230 mesh or 230−400 mesh) was used for column chromatography, and Riedel-de Haen DC-Mikrokarten SI F 37341 were used for TLC. ^{1}H-NMR spectra were recorded with a Bruker AC 200 spectrometer in the indicated solvents (TMS as internal standard); the values of the chemical shifts are expressed in ppm and coupling constants (J) in hertz (Hz). Mass spectra (EI, 70 eV) were recorded on a VG 70-250S spectrometer. NMR spectra and elemental analyses were performed by the Dipartimento Farmaco Chimico Tecnologico, Università di Siena. Mass spectra were performed by Centro di Analisi e Determinazioni Strutturali, Università di Siena.

Ethyl 4-(2-Fluorophenyl)-3-methylquinoline-2-carboxylate (10). To a solution of 2-amino-2′-fluorobenzophenone (**8**) (6.5 g, 30 mmol) in dry methanol (150 mL) with 2-oxobutyric acid (3.4 g, 33 mmol) was added a 30% solution of sodium methoxide (10 mL, 54 mmol), and the resulting mixture was heated at reflux for 6 days. After removal of the solvent *in vacuo*, the residue was partitioned between ether−THF and water; the organic layer was discarded, while the aqueous solution was acidified with acetic acid and extracted with ethyl acetate. The extracts were dried over sodium sulfate and evaporated to dryness *in vacuo* to obtain a thick oil which crystallized on standing. By washing with ether, pure **9** was obtained (2.4 g, 8.5 mmol) and dissolved in dry ethanol (100 mL), and phosphorus oxycloride (5 mL, 546 mmol) was added. The resulting mixture was heated at reflux overnight and the solvent evaporated *in vacuo*; the residue was diluted with water, basified by addition of solid Na_2CO_3, and finally extracted with dichloromethane. The extracts were washed with water, dried over sodium sulfate, and evaporated *in vacuo* to give **10** (2.6 g, yield 28%) as colorless crystals. An analytical sample recrystallized from *n*-hexane melted at 107−108 °C: ^{1}H-NMR ($CDCl_3$) 1.49 (t, $J = 7.0$, 3H), 2.35 (s, 3H), 4.56 (q, $J = 7.0$, 2H), 7.19−7.58 (m, 6H), 7.66−7.74 (m, 1H), 8.21 (d, $J = 8.5$, 1H). Anal. ($C_{19}H_{16}NO_2F$) C, H, N.

Ethyl 3-(Bromomethyl)-4-(2-fluorophenyl)quinoline-2-carboxylate (11). A mixture of **10** (0.8 g, 2.6 mmol) in CCl_4 (50 mL) with *N*-bromosuccinimide (0.45 g, 2.5 mmol) and dibenzoyl peroxide (0.1 g, 0.4 mmol) was heated at reflux overnight, the solvent was evaporated *in vacuo*, and the residue was diluted with a small portion of the same solvent and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography by eluting with dichloromethane. Thus pure **11** was obtained as colorless

crystals (0.84 g, yield 83%). Recrystallization from *n*-hexane gave an analytical sample melting at 91−92 °C: ^{1}H-NMR ($CDCl_3$) 1.52 (t, J = 7.2, 3H), 4.51−4.68 (m, 3H), 5.00 (d, J = 10.0, 1H), 7.30−7.42 (m, 4H), 7.49−7.64 (m, 2H), 7.73−7.81 (m, 1H), 8.25 (d, J = 8.3, 1H). Anal. ($C_{19}H_{15}NO_2BrF$) C, H, N.

(±)-1,2-Dihydro-9-(2-fluorophenyl)-2-(1-methylpropyl)-3*H*-pyrrolo[3,4-*b*]quinolin-3-one (3b). A mixture of **11** (0.23 g, 0.59 mmol) in ethanol (20 mL) and *sec*-butylamine (0.16 mL, 1.6 mmol) with K_2CO_3 (0.5 g, 3.6 mmol) was refluxed for 1 h and the solvent then removed *in vacuo*. The residue was partitioned between $CHCl_3$ and water, and the organic layer then was washed with water, dried over sodium sulfate, and concentrated *in vacuo*. Purification of the residue by column chromatography, eluting with CH_2Cl_2−ethyl acetate (8:2), gave **3b** as a white powder: ^{1}H-NMR ($CDCl_3$) 0.86−0.95 (m, 3H), 1.24−1.30 (m, 3H), 1.55−1.71 (m, 2H), 4.10−4.34 (m, 2H), 4.53−4.71 (m, 1H), 7.28−7.43 (m, 3H), 7.50−7.82 (m, 4H), 8.46 (d, J = 8.4, 1H).

General Procedure for the Synthesis of Compounds 4 and 6. Method A. To a solution of the suitable carboxylic acid (**9**, **12**[10] or **20**) (1.0 mmol) in CH_2Cl_2 (20 mL) cooled at 0−5 °C was added $SOCl_2$ (1.0 mL), and the resulting solution was stirred at room temperature for 30 min. The solvent was distilled under reduced pressure, and excess $SOCl_2$ was removed by distillation with toluene. The residue was diluted with CH_2Cl_2 (20 mL) and cooled at 0−5 °C, and the suitable primary amine (30 mmol) was added (in the case of compounds **4c**−**e** 1.1 mmol of the suitable primary amine and 20 mmol of triethylamine were used). The resulting mixture was stirred overnight at room temperature, washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was recrystallized from the suitable solvent, and the corresponding secondary amide (**4a**−**e** or **6a**) was obtained in analytically pure form.

(±)-3-Methyl-*N*-(1-methylpropyl)-4-phenylquinoline-2-carboxamide (4a): ^{1}H-NMR ($CDCl_3$) 1.03 (t, J = 7.5, 3H), 1.32 (d, J = 6.9, 3H), 1.57−1.79 (m, 2H), 2.56 (s, 3H), 4.07−4.21 (m, 1H), 7.19−7.69 (m, 8H), 7.88 (d, J = 8.1, 1H), 8.09 (d, J = 8.3, 1H).

(±)-4-(2-Fluorophenyl)-3-methyl-*N*-(1-methylpropyl)-quinoline-2-carboxamide (4b): ^{1}H-NMR ($CDCl_3$) 1.03 (t, J = 7.4, 3H), 1.30−1.33 (m, 3H), 1.60−1.76 (m, 2H), 2.59 (s, 3H), 4.05−4.20 (m, 1H), 7.16−7.71 (m, 7H), 7.88 (br s, 1H), 8.10 (d, J = 8.4, 1H).

3-Methyl-4-phenyl-*N*-(phenylmethyl)quinoline-2-carboxamide (4c): ^{1}H-NMR ($CDCl_3$) 2.60 (s, 3H), 4.73 (d, J = 6.2, 2H), 7.20−7.69 (m, 13H), 8.05 (d, J = 8.4, 1H) 8.47 (t, J = 6.2, 1H).

***N*-[(4-Chlorophenyl)methyl]-3-methyl-4-phenylquinoline-2-carboxamide (4d):** ^{1}H-NMR ($CDCl_3$) 2.59 (s, 3H), 4.69 (d, J = 6.3, 2H), 7.21−7.69 (m, 12H), 8.06 (d, J = 8.4, 1H) 8.53 (t, J = 6.3, 1H).

***N*-[(4-Chlorophenyl)methyl]-4-(2-fluorophenyl)-3-methylquinoline-2-carboxamide (4e):** ^{1}H-NMR ($CDCl_3$) 2.63 (s, 3H), 4.69 (d, J = 6.4, 2H), 7.17−7.71 (m, 11H), 8.07 (d, J = 8.3, 1H) 8.54 (t, J = 6.4, 1H).

(±)-4-Methyl-*N*-(1-methylpropyl)-1-phenylisoquinoline-3-carboxamide (6a): ^{1}H-NMR ($CDCl_3$) 0.97 (t, J = 7.4, 3H), 1.24 (d, J = 6.7, 3H), 1.52−1.74 (m, 2H), 3.20 (s, 3H), 4.05−4.20 (m, 1H), 7.53−7.81 (m, 7H), 8.11 (d, J = 8.4, 1H), 8.28 (d, J = 8.6, 1H), 8.34 (br s, 1H).

Method B. To a solution of the appropriate secondary amide (**4a,b,d,e** or **6a**) (0.5 mmol) in anhydrous DMF (5 mL) with CH_3I (0.5 mL, 8.0 mmol) cooled at 0 °C under argon atmosphere was added NaH (0.05 g, 2.1 mmol). The resulting mixture was stirred at 0 °C for 30 min. The reaction mixture was poured into ice−water, neutralized with 1 N HCl, and extracted with $CHCl_3$ (3 × 25 mL). The combined organic extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure to give the corresponding tertiary amide, which was purified by recrystallization from the suitable solvent (**4f,i**) or by column chromatography by eluting with $CHCl_3$−ethyl acetate (8:2) (**4g,j** or **6b**). (Since the amide nitrogen of these compounds bears two different substituents, their ^{1}H-NMR spectra show the presence of two different rotamers in equilibrium. For the sake of simplification, the integral intensities have not been given.)

(±)-*N*,3-Dimethyl-*N*-(1-methylpropyl)-4-phenylquinoline-2-carboxamide (4f): ^{1}H-NMR ($CDCl_3$) 0.86 (t, J = 7.4), 1.03 (t, J = 7.4), 1.22−1.69 (m), 2.20 (s), 2.23 (s), 2.72 (s), 3.04 (s), 3.40−3.58 (m), 4.82−4.92 (m), 7.26−7.68 (m), 8.07−8.14 (m).

(±)-*N*,3-Dimethyl-4-(2-fluorophenyl)-*N*-(1-methylpropyl)quinoline-2-carboxamide (4g): ^{1}H-NMR ($CDCl_3$) 0.79−0.92 (m), 0.98−1.09 (m), 1.19−1.71 (m), 2.22−2.25 (m), 2.71 (s), 3.04 (s), 3.41−3.49 (m), 4.85−4.95 (m), 7.22−7.70 (m), 8.09−8.15 (m); MS m/z calcd for $C_{22}H_{23}N_2OF$ (M^+) 350.1794, found 350.1796.

***N*-[(4-Chlorophenyl)methyl]-*N*,3-dimethyl-4-phenylquinoline-2-carboxamide (4i):** ^{1}H-NMR ($CDCl_3$) 2.19 (s), 2.20 (s), 2.83 (s), 3.13 (s), 4.43 (s), 4.83 (s), 7.20−7.69 (m), 8.07−8.14 (m).

***N*-[(4-Chlorophenyl)methyl]-*N*,3-dimethyl-4-(2-fluorophenyl)quinoline-2-carboxamide (4j):** ^{1}H-NMR ($CDCl_3$) 2.20 (s), 2.22 (s), 2.81 (s), 3.13 (s), 4.41 (s), 4.82 (AB q, J = 14.6), 7.22−7.71 (m), 8.08−8.15 (m).

(±)-*N*,4-Dimethyl-*N*-(1-methylpropyl)-1-phenylisoquinoline-3-carboxamide (6b): ^{1}H-NMR ($CDCl_3$) 0.80 (t, J = 7.3), 1.01 (t, J = 7.4), 1.19−1.73 (m), 2.65 (s), 2.67 (s), 2.69 (s), 3.00 (s), 3.38−3.53 (m), 4.79−4.98 (m), 7.46−7.78 (m), 8.04−8.11 (m).

Method C. A solution of the appropriate carboxylic acid (**12**[10] or **20**) (1.0 mmol) in CH_2Cl_2 (20 mL) with $SOCl_2$ (1.0 mL, 13.7 mmol) was stirred at room temperature for 30 min, and the solvent was evaporated under reduced pressure. The excess of $SOCl_2$ was removed by azeotropic distillation with toluene and the residue diluted with CH_2Cl_2 (20 mL) and then cooled to 0−5 °C. To the resulting mixture the suitable secondary amine (1.1 mmol) (in the case of compound **4k**, *N*-methyl-4-chloroaniline hydrochloride was used) and triethylamine (1.4 mL, 10 mmol) were added in sequence. The reaction mixture was stirred overnight at room temperature, washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by column chromatography, eluting with CH_2Cl_2−ethyl acetate (8:2), gave the corresponding tertiary amide (**4h,k,l** or **6c**). Compounds **4k,l** were recrystallized from the suitable solvent. (Since the amide nitrogen of these compounds bears two different substituents, their ^{1}H-NMR spectra show the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given).

***N*,3-Dimethyl-4-phenyl-*N*-(phenymethyl)quinoline-2-carboxamide (4h):** ^{1}H-NMR ($CDCl_3$) 2.19 (s), 2.23 (s), 2.83 (s), 3.15 (s), 4.48 (s), 4.87 (s), 7.21−7.70 (m), 8.12 (d, J = 8.6).

***N*-(4-Chlorophenyl)-*N*,3-dimethyl-4-phenylquinoline-2-carboxamide (4k):** ^{1}H-NMR ($CDCl_3$) 2.14 (s), 2.31 (s), 3.30 (s), 3.56 (s), 7.07−7.63 (m), 7.98 (d, J = 8.4), 8.15 (d, J = 8.4).

***N*,3-Dimethyl-*N*-(4-methoxyphenyl)-4-phenylquinoline-2-carboxamide (4l):** ^{1}H-NMR ($CDCl_3$) 2.14 (s), 2.32 (s), 3.27 (s), 3.54 (s), 3.66 (s), 3.84 (s), 6.61−6.65 (m), 6.97−7.61 (m), 8.00 (d, J = 8.4), 8.16 (d, J = 8.4).

***N*,4-Dimethyl-1-phenyl-*N*-(phenylmethyl)isoquinoline-3-carboxamide (6c):** ^{1}H-NMR ($CDCl_3$) 2.69 (s), 2.70 (s), 2.80 (s), 3.10 (s), 4.44 (s), 4.84 (s), 7.25−7.79 (m), 8.03−8.11 (m).

Ethyl 1-Phenyl-4-[[(trifluoromethyl)sulfonyl]oxy]isoquinoline-3-carboxylate (14). To a solution of **13** (0.96 g, 3.3 mmol) in anhydrous pyridine (15 mL) cooled at 0 °C was added dropwise triflic anhydride (0.66 mL, 3.9 mmol). The resulting mixture was stirred for 5 min at 0 °C and overnight at room temperature. The reaction mixture was poured into ice−water and the precipitate collected by filtration, washed with water, and dried to give pure **14** (1.3 g, yield 93%). An analytical sample was recrystallized from methanol (mp 129−130 °C): ^{1}H-NMR ($CDCl_3$) 1.47 (t, J = 6.9, 3H), 4.55 (q, J = 6.9, 2H), 7.53−7.56 (m, 3H), 7.70−7.79 (m, 3H), 7.89−7.97 (m, 1H), 8.18−8.27 (m, 2H). Anal. ($C_{19}H_{14}NO_5SF_3$) C, H, N.

Ethyl 4-Methyl-1-phenylisoquinoline-3-carboxylate (15). A solution of **14** (0.64 g, 1.5 mmol) in anhydrous DMF with LiCl (0.35 g, 8.3 mmol) and tetramethyltin (1.0 mL, 7.2 mmol) was stirred under an argon stream for 10 min, and $Pd(PPh_3)Cl_2$ (0.11 g, 0.16 mmol) was added. The mixture was heated

at 140 °C for 40 min, poured into ice−water, and extracted with CH_2Cl_2 (5 × 15 mL). The combined organic extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography by eluting with CH_2Cl_2−ethyl acetate (9:1), and analytically pure **15** (0.42 g, yield 96%) was obtained as a pale yellow oil: ^{1}H-NMR ($CDCl_3$) 1.45 (t, J = 6.9, 3H), 2.85 (s, 3H), 4.50 (q, J = 6.9, 2H), 7.48−7.83 (m, 7H), 8.12 (d, J = 8.1, 1H), 8.18 (d, J = 8.5, 1H). Anal. ($C_{19}H_{17}NO_2$) C, H, N.

Ethyl 4-(Bromomethyl)-1-phenylisoquinoline-3-carboxylate (16). A mixture of **15** (0.18 g, 0.62 mmol) in CCl_4 (20 mL) with *N*-bromosuccinimide (0.11 g, 0.62 mmol) and dibenzoyl peroxide (0.03 g, 0.12 mmol) was heated at reflux for 2 h and 15 min, the solvent was evaporated *in vacuo*, and the residue was diluted with a small portion of the same solvent and filtered. The filtrate was concentrated *in vacuo* and the residue purified by column chromatography by eluting with dichloromethane. Thus, pure **16** was obtained as colorless crystals (0.22 g, yield 96%); recrystallization from *n*-hexane gave an analytical sample melting at 109−110 °C: ^{1}H-NMR ($CDCl_3$) 1.48 (t, J = 7.3, 3H), 4.54 (q, J = 7.2, 2H), 5.30 (s, 2H), 7.51−7.73 (m, 6H), 7.85−7.93 (m, 1H), 8.18 (d, J = 8.4, 1H), 8.32 (d, J = 8.6, 1H). Anal. ($C_{19}H_{16}NO_2Br$) C, H, N.

(±)-1,2-Dihydro-2-(1-methylpropyl)-5-phenyl-3*H*-pyrrolo[3,4-*c*]isoquinolin-3-one (5a). A mixture of **16** (0.11 g, 0.30 mmol) in ethanol (20 mL) with *sec*-butylamine (0.18 mL, 1.8 mmol) was heated at reflux for 2 h, and then the volatiles were removed under reduced pressure. Purification by column chromatography of the residue, using CH_2Cl_2−ethyl acetate (8:2) as eluent, gave pure **5a** as white crystals (0.086 g, yield 90%): ^{1}H-NMR ($CDCl_3$) 0.96 (t, J = 7.4, 3H), 1.37 (d, J = 6.8, 3H), 1.67−1.82 (m, 2H) 4.56−4.76 (m, 3H), 7.49−7.53 (m, 3H), 7.62−7.74 (m, 3H), 7.80−7.88 (m, 1H), 7.96 (d, J = 8.0, 1H), 8.23 (d, J = 8.5, 1H).

1,2-Dihydro-5-phenyl-2-(phenylmethyl)-3*H*-pyrrolo-[3,4-*c*]isoquinolin-3-one (5b). Starting from **16** (0.11 g, 0.30 mmol), **5b** was prepared as described for **5a**, by using benzylamine (0.20 mL, 1.8 mmol) instead of *sec*-butylamine: ^{1}H-NMR ($CDCl_3$) 4.61 (s, 2H), 4.97 (s, 2H), 7.26−7.87 (m, 13H), 8.23 (d, J = 8.6, 1H).

Ethyl 4-(3-Hydroxypropyn-1-yl)-1-phenylisoquinoline-3-carboxylate (17). A mixture of **14** (0.43 g, 1.0 mmol), propargyl alcohol (0.60 mL, 10.3 mmol), triethylamine (1.0 ml, 7.2 mmol), cuprous iodide (0.016 g, 0.084 mmol), and anhydrous DMF (10 mL) was degassed with argon for 10 min. Pd-$(PPh_3)Cl_2$ (0.071 g, 0.1 mmol) was added, and the resulting mixture was heated to 60−65 °C for 45 min, poured into ice−water, and extracted with $CHCl_3$ (3 × 20 mL). The combined organic extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography, eluting with CH_2Cl_2−ethyl acetate (8:2) to give **17** (0.21 g, yield 63%). An analytical sample was recrystallized from diethyl ether−*n*-hexane (mp 86−87 °C): ^{1}H-NMR (DMSO-d_6) 1.36 (t, J = 7.3, 3H), 4.40 (q, J = 7.2, 2H), 4.51 (d, J = 6.0, 2H), 5.54 (t, J = 6.2, 1H), 7.56−7.71 (m, 5H), 7.78−7.86 (m, 1H), 7.97−8.11 (m, 2H), 8.48 (d, J = 8.2, 1H). Anal. ($C_{21}H_{17}NO_3$) C, H, N.

Ethyl 4-(3-Hydroxypropyl)-1-phenylisoquinoline-3-carboxylate (18). A solution of **17** (0.14 g, 0.42 mmol) in ethanol (20 mL) with 10% Pd/C (0.07 g) was hydrogenated in a Parr apparatus at 25 psi at room temperature for 4 h. The catalyst was removed by filtration and washed with hot ethanol and the filtrate concentrated under reduced pressure. Purification of the residue by chromatography with $CHCl_3$−ethyl acetate (8:2) as eluent gave **18** (0.11 g, yield 78%) as a colorless oil which crystallized on standing. An analytical sample was obtained from diethyl ether−*n*-hexane (mp 62−63 °C): ^{1}H-NMR (DMSO-d_6) 1.34 (t, J = 7.1, 3H), 1.76−1.90 (m, 2H), 3.15−3.23 (m, 2H), 3.56 (q, J = 5.8, 2H), 4.39 (q, J = 7.1, 2H), 4.64 (t, J = 5.3, 1H), 7.54−7.77 (m, 6H), 7.88−8.04 (m, 2H), 8.34 (d, J = 8.5, 1H). Anal. ($C_{21}H_{21}NO_3$) C, H, N.

(*z*)-Ethyl 4-(3-Hydroxypropen-1-yl)-1-phenylisoquinoline-3-carboxylate (19). A solution of **17** (0.19 g, 0.57 mmol) in ethanol (30 mL) with 5% PdS/C (0.1 g) was hydrogenated in a Parr apparatus at 20 psi at room temperature for 2 h. The catalyst was removed by filtration and washed with hot ethanol and the filtrate concentrated under reduced pressure. Purification of the residue by chromatography with CH_2Cl_2−ethyl acetate (8:2) as eluent gave **19** (0.14 g, yield 74%) as a colorless oil which crystallized by treatment with diethyl ether−*n*-hexane. An analytical sample was recrystallized from diethyl ether−*n*-hexane (mp 101−102 °C): ^{1}H-NMR (DMSO-d_6) 1.30 (t, J = 7.0, 3H), 3.65−3.72 (m, 2H), 4.31 (q, J = 7.2, 2H), 4.65 (t, J = 5.0, 1H), 6.09−6.21 (m, 1H), 6.84 (d, J = 11.3, 1H), 7.56−7.80 (m, 6H), 7.86−7.93 (m, 1H), 8.05 (d, J = 8.3, 1H), 8.11 (d, J = 8.3, 1H). Anal. ($C_{21}H_{19}NO_3$) C, H, N.

Method D. To a solution of the suitable alcohol (**18** or **19**) (0.4 mmol) in CH_2Cl_2 (20 mL) cooled at 0−5 °C was added, $SOCl_2$ (1.0 mL, 13.7 mmol) and the mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the excess of $SOCl_2$ was removed by azeotropic distillation with benzene. The residue was dissolved with ethanol (20 mL), *sec*-butylamine (3.0 mL, 29.7 mmol) was added, and the resulting mixture was heated to reflux overnight. The volatiles were removed under reduced pressure, the residue was diluted with absolute ethanol (30 mL), and to the resulting mixture was added a 30% MeONa/MeOH solution (1.0 mL, 5.4 mmol). After 2 h of heating at reflux, the reaction mixture was cooled in an ice−water bath and neutralized with 1 N ethanolic HCl. The solvent was removed under reduced pressure, the residue was diluted with CH_2Cl_2 (30 mL), and $SOCl_2$ (1.0 mL, 13.7 mmol) was added. After 30 min of stirring at room temperature, the volatiles were distilled under reduced pressure, benzene (20 mL) was added, and the solvent was evaporated. The residue was partitioned between CH_2Cl_2 (30 mL) and a saturated solution of $NaHCO_3$ (20 mL). The organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by column chromatography using $CHCl_3$−ethyl acetate (8:2) as eluent gave the corresponding lactam (**5c** or **5d**) which was recrystallized from the suitable solvent.

(±)-4-(1-Methylpropyl)-7-phenyl-1,2,3,4-tetrahydro-5*H*-azepino[3,4-*c*]isoquinolin-5-one (5c): ^{1}H-NMR ($CDCl_3$) 1.01 (t, J = 7.3, 3H), 1.27 (d, J = 6.8, 3H), 1.50−1.70 (m, 2H), 2.04−2.18 (m, 2H), 3.19−3.33 (m, 4H), 4.87−4.98 (m, 1H), 7.46−7.61 (m, 4H), 7.70−7.80 (m, 3H), 8.13−8.22 (m, 2H).

(±)-3,4-Dihydro-4-(1-methylpropyl)-7-phenyl-5*H*-azepino[3,4-*c*]isoquinolin-5-one (5d): ^{1}H-NMR ($CDCl_3$) 0.97 (t, J = 7.4, 3H), 1.24 (d, J = 6.8, 3H), 1.52−1.67 (m, 2H), 3.67 (d, J = 7.1, 2H), 4.73−4.91 (m, 1H), 6.52−6.64 (m, 1H), 7.43−7.65 (m, 5H), 7.74−7.82 (m, 3H), 8.13−8.20 (m, 2H).

3,4-Dihydro-7-phenyl-4-(phenylmethyl)-5*H*-azepino-[3,4-*c*]isoquinolin-5-one (5e) (Method E). To a solution of **19** (0.08 g, 0.24 mmol) in CH_2Cl_2 (20 mL) cooled at 0−5 °C was added, $SOCl_2$ (1.0 mL, 13.7 mmol) and the mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the excess of $SOCl_2$ was removed by azeotropic distillation with benzene. The residue was dissolved with ethanol (20 mL), benzylamine (1.0 mL, 9.1 mmol) was added, and the resulting mixture was heated at reflux overnight. The volatiles were removed under reduced pressure, the residue was diluted with toluene, 2-hydroxypyridine (0.05 g, 0.52 mmol) was added, and the resulting mixture was heated at reflux for an additional 24 h. The solvent was removed under reduced pressure and the residue purified by chromatography. Elution with $CHCl_3$−ethyl acetate (8:2) gave pure **5e**: ^{1}H-NMR ($CDCl_3$) 3.76 (d, J = 7.0, 2H), 4.89 (s, 2H), 6.29−6.40 (m, 1H), 7.26−7.66 (m, 10H), 7.75−7.82 (m, 3H), 8.13−8.20 (m, 2H).

4-Methyl-1-phenylisoquinoline-3-carboxylic Acid (20). A solution of **15** (0.41 g, 1.4 mmol) in ethanol (30 mL) with 3 N NaOH (10 mL, 30 mmol) was heated at reflux for 20 min and the solvent evaporated under reduced pressure. The residue was diluted with cold water (30 mL) and acidified with 3 N HCl (pH 5−6). The precipitate was extracted with $CHCl_3$ (3 × 10 mL), and the combined organic extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Thus, **20** (0.33 g, yield 89%) was obtained as a white solid and used in the next steps without further

purification: ^{1}H-NMR ($CDCl_3$) 3.25 (s, 3H), 7.55−7.75 (m, 6H), 7.85−7.93 (m, 1H), 8.20 (d, J = 8.6, 1H), 8.36 (d, J = 8.4, 1H).

Method F. To a solution of the suitable imidoyl chloride (**21a** or **21b**) (0.41 mmol) in anhydrous DMF (5 mL) with PPh_3 (0.026 g, 0.1 mmol) under a CO atmosphere were added $PdAc_2$ (0.011 g, 0.049 mmol) and N-methylbenzylamine (0.27 mL, 2.1 mmol) in sequence. The resulting mixture was heated at 80−90 °C for 7 h, poured into ice−water, and extracted with $CHCl_3$ (4 × 20 mL). The organic layer was washed with water, dried over sodium sulfate, and evaporated under reduced pressure to give an oil residue which was purified by column chromatography. Elution with *n*-hexane−ethyl acetate (65:35) gave the corresponding amide (**7a** or **7b**). (Since the amide nitrogen of these compounds bears two different substituents, their ^{1}H-NMR spectra show the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given.)

7,8-Dihydro-*N*-methyl-*N*-(phenylmethyl)benzo[*k*]-phenanthridine-6-carboxamide (7a): ^{1}H-NMR ($CDCl_3$) 2.82 (s), 2.86−2.93 (m), 3.15 (s), 4.50 (s), 4.87 (s), 7.25−7.43 (m), 7.52−7.75 (m), 7.90−7.98 (m), 8.13−8.19 (m), 8.43−8.49 (m).

6,7-Dihydro-*N*-methyl-*N*-(phenylmethyl)-[1]benzoxepino[4,5-*c*]quinoline-8-carboxamide (7b): ^{1}H-NMR ($CDCl_3$) 2.72−3.11 (m), 3.15 (s), 4.42−4.70 (m), 4.87 (AB q, J = 14.7), 7.25−7.59 (m), 7.67−7.76 (m), 8.03−8.18 (m).

Computational Procedure. The structures of all compounds studied were fully optimized by means of molecular orbital calculations (AM1),[19] using the MOPAC 6.0 (QCPE 455) program, by applying the molecular mechanics correction to the amide bond. The three-dimensional structure of **1**[4] and of compound **5d**, solved by X-ray crystallography in our laboratory (see text), were taken as the input for the AM1 optimization. The starting geometry of the other derivatives considered were constructed within the Chemnote module of Quanta 4.1 program. Enantiomer *R* was arbitrarily chosen for those compounds which show an asymmetric carbon in their structures. Both *R* and *S* enantiomers of **1** have been found to be nearly equipotent toward PBR ((*R*)-**1**, IC_{50} = 9 nM; (*S*)-**1**, IC_{50} = 19 nM; (*R*,*S*)-**1**, IC_{50} = 9 nM).[3]

Conformational analysis was performed for the parent compounds **4b**,**g** and **6a**, and for **1**. The most important conformational freedom is the rotation around the torsional angle ϕ (see Chart 1), which modulates the space orientation of the amide function. Uniform scanning of this dihedral angle was performed, in a rigid manner, in steps of 10° on the fully optimized structures. The relaxation of the other degrees of freedom does not affect the qualitative nature of the potential energy trends, although it usually reduces the barrier to the rotation between conformers.

Conformational preferences in the region of the absolute AM1 minimum were studied at the *ab initio* level (RHF/6-31G*); the Gaussian94 program was used.[20]

Molecular Superimposition. Compounds **4f**,**h**, and **6c**, the most structurally different ligands which show high affinity for PBR, were chosen for the construction of the reference supermolecule and were superimposed, by a rigid fit procedure, on the basis of their common quinoline or isoquinoline nucleus. All of the other compounds were satisfactorily matched on the supermolecule by using their global minimum conformer or conformers whose energy is only 1 kcal/mol above the global minimum.

The ad-hoc-defined size and shape descriptor, V_d, is computed according to the following formulas: $V_d = (V_{in} - V_{out})/V^{sup}$ where V^{sup} is the resultant van der Waals volume of the reference supermolecule. V_{in} and V_{out} are, respectively, the intersection and the outer van der Waals volume of the ligands considered in relation to the supermolecule.

The QUANTA 4.1[22] molecular modeling software implemented on a HP 720 personal workstation was used for molecular comparisons, matching, and computation of van der Waals volumes.

X-Ray Crystallography. A single crystal of **5d** was submitted to X-ray data collection on a Siemens P4 four-circle diffractometer with graphite monochromated Mo Kα radiation. The $\omega/2\theta$ scan technique was used.

5d: $C_{23}H_{22}N_2O$ (mol wt 342.4), a colorless single crystal, dimensions 0.2 × 0.3 × 0.2 mm was used for data collection; monoclinic; *Pc* space group; a = 8.825(2) Å, b = 9.261(2) Å, c = 11.719(2) Å, β = 108.40(3)°, V = 908.8(3) Å^3, Z = 2, D_c = 1.25 g/cm^3. A total of 3195 unique reflections (R_{int} = 0.011) were collected at 22 °C, of which 2762 were observed with $F_o > 4\sigma(F_o)$. No absorption correction was applied. The structure was solved by direct methods of the SHELXTL PC package.[23]

As **5d** crystallizes in a polar space group, polar axis restraints were applied by Flack and Schwarzenbach method.[24] Atomic scattering factors including f' and f'' were taken from ref 25. The refinement was carried out by full-matrix anisotropic least-squares on F^2 for all reflections for non-H atoms by using SHELXL-93 program.[26] Function $\sum w(F_o^2 - F_c^2)^2$ was minimized with weighing scheme $w = 1/[\sigma^2(F_o)2 + aP^2 + bP]$, where $P = (F_o^2 + 2F_c^2)/3$, a = 0.0570 and b = 0.1891.

The final refinement converged to R1 = 0.054 and wR2 = 0.1103, goodness of fit = 1.037 for all data. R1 refers to the conventional *R* factor based on *F*, while wR2 and goodness of fit are based on F^2. *R* factors based on F^2 are statistically about twice as large as those based on *F*.[26]

Min and max height in last $\Delta\rho$ map were −0.19 and 0.236 e Å^{-3}, respectively.

During the refinement a statistical disorder was shown by the CH and by one CH_3 of the pendant substituent. For these atoms two different positions were refined with the sum of the site occupation factors (sof) fixed at 1. Refined values of sof equal to 0.849(5) and 0.151(5) were found for each of the two positions.

Full crystallographic details will be given elsewhere.

***In Vitro* Binding Assays.** Male CRL: CD(SD)BR (Charles River Italia, Calco, CO, Italy) rats were killed by decapitation, their brains were rapidly dissected into the various areas and stored at −80°C until the day of assay. The binding assays were performed as described by Cantoni et al.[27] The frozen cortices were homogenized in 50 vols of ice-cold phosphate-buffered saline (PBS) 50 mM, pH 7.4 containing 100 mM NaCl, using an Ultra Turrax TP-1810 (2 × 20 sec). The homogenate was centrifuged for 10 min at 1000*g* at 4° C (Beckman model J-21B refrigerated centrifuge). The pellet was washed three more times by resuspension in the same volume of fresh buffer and centrifuged again at 50000*g* for 10 min. The pellet obtained was finally resuspended in the incubation buffer (PBS) just before the binding assay.

[^{3}H]-**1** (s.a. 86.4 Ci/mmol, NEN) binding was assayed in a final incubation volume of 1.0 mL, consisting of 0.5 mL membrane suspension, 0.5 mL of [^{3}H]ligand and 20 μl of displacing agent or solvent. Tissue concentration and [^{3}H]-**1** final concentration were 2.3 mg tissue/sample and 1 nM, respectively.

Incubations (120 min at 4° C) were stopped by rapid filtration under vacuum through GF/B fiber filters which were then washed with 12 mL (3 × 4 mL) of ice-cold PBS using a Brandel M-48RP cell harvester.

Nonspecific binding was assayed in the presence of **1** (1 μM).

Dried filters were immersed in vials containing 4 mL of Filter Count (Packard) liquid scintillation cocktail for the measurement of trapped radioactivity with a Packard LKB 1214 RACKBETA liquid scintillation spectrometer at a counting efficiency of about 60%. The concentration of the test compounds that inhibited [^{3}H]ligand binding by 50% (IC_{50}) was determined using the Allfit [28] program with 6 concentrations of the displacers, each performed in triplicate.

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